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METABOLISM OF FENTHION BY AQUATIC MICROBIAL COMMUNITIES

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Abstract - The microbial metabolism of the mosquito control agent, fenthion, has been studied in shake flask systems containing water and sediment from a salt marsh. The usefulness of this information in describing the fate of fenthion in microcosms and in a field dosing experiment was detemined. Our results show that microbial communities associated with the sediment, the presence of invertebrate animals in the sediment bed, and the anaerobic conditions of the sediment contribute significantly to the fate of fenthion under natural conditions.

INTRODUCTION

Recent developments in hazard assessment procedures have centered on improvements in exposure concentration estimations, particularly in those that extrapolate laboratory information to field situations. Metabolism by microorganisms is frequently one of the major processes that control the fate of a pesticide in the environment. Although a number of standardized tests for establishing susceptibility to microbial metabolism exist, few investigations (ref. 1 and 2) have attempted to relate the resulting information to the complex environmental conditions found in the field.

The objective of our study was to further clarify these relationships. Specifically, we proposed to characterize the environmental factors that control the microbial metabolism of the organophosphate pesticide, fenthion (Baytex), and then determine the relevance of this information in describing the fate of fenthion in microcosms (laboratory surrogates to the field) and field dosing experiments. Because fenthion is extensively applied to coastal areas to control mosquito populations, our studies dealt with the fate of this pesticide in an estuarine salt marsh.

FLASK STUDIES

The ability of microorganisms to metabolize fenthion was established in laboratory shake flask studies. Inocula for the flask studies consisted of water and detrital sediment collected from a local salt marsh. The detrital sediment was light, fluffy, high organic particulates composed largely of decaying plant material and heavily colonized with bacteria, algae, and protozoa; sand was excluded. Flasks containing water or water-sediment slurries (500 mg/ℓ dry weight) were spiked with fenthion to a final concentration of approximately 200 µg/l, and incubated on a rotary shaker, in the dark, at 25° C. Changes in the concentration of fenthion (figure 1) were followed by periodically removing samples from the flasks, extracting the samples with hexane or acetonitrile, and analyzing the extracts by gas chromatography.

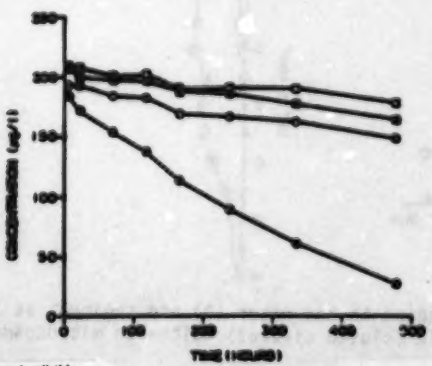

Figure 1. Disappearance rates of fenthion in formalin-sterile (open symbols) and nonsterile (closed symbols) flasks containing water (squares) and sediment (circles).

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Fenthion disappeared slowly (significantly different [$\alpha = 0.05$] from a slope of zero; half life = > 30 days) in sterile flasks, indicating little susceptibility to hydrolysis. The slow loss in nonsterile water (not significantly different [$\alpha = 0.05$] from the sterile water; half life = > 30 days) suggested that microbial populations contained therein either were not able to metabolize fenthion or the biomass of degrading microorganisms was not sufficient to cause significant loss during the time course of the experiment. The rapid loss of fenthion in the presence of nonsterile sediment (half life = 7.4 days) indicates that the microbial populations associated with the sediment surface were capable of degrading the pesticide.

Additional flask experiments with radiolabeled fenthion showed the production of polar (relative to fenthion) products, the production of bound residues (radioactivity associated with sediment that could not be extracted with conventional solvents), and the production of very small amounts of radiolabeled carbon dioxide. These results are consistent with the idea that the disappearance of fenthion in the presence of sediments is due to the metabolic activity of bacteria associated with the sediment.

Several other environmental factors affected the fate of fenthion in saltmarsh environments, as shown by additional flask studies. Biodegradation rates were proportional to detrital sediment concentration and the microorganisms responsible for the degradation activity associated with the sediments could not be released into the water if vigorously shaken with the sediment. Degradation was much slower when oxygen was removed by sparging the sediment-water slurries in the flasks with nitrogen gas. The pesticide was also photolabile, having a half life in water of 6.2 days when exposed directly to sunlight.

MICROCOSM STUDIES

As a means of verifying the importance of sediment in the degradation of fenthion under more natural conditions, the fate of fenthion in microcosms was determined. Microcosms consisted of 3-ℓ vessels (15 cm in diameter) containing an intact core of sediment (detrital sediment mixed with sand) from the salt marsh; 2 1/2 ℓ of seawater from the marsh provided the water column. Complete mixing of the water column without resuspension of the sediment was accomplished with a bent glass rod attached to a constant rpm motor. The microcosms were exposed to a 12:12 hour light:dark cycle, using fluorescent lighting. Experiments commenced by spiking the water column with fenthion to a final concentration of 200 µg/l. Water samples were removed periodically and the concentration of the pesticide determined. After 170 hours of incubation, the water in the microcosms was siphoned off and the sediment bed was sliced into 0.5 cm layers (by frozen core fractionation), extracted, and the total concentration (dissolved and sorbed) of fenthion determined. Porosity and the concentration of detrital sediment were also determined for each layer. Microcosms treated with formalin were used to separate biotic form abiotic effects.

The changes in concentration of fenthion in the water column and the distribution of fenthion in the sediment bed after 170 hours of incubation are shown in figures 2a and b respectively.

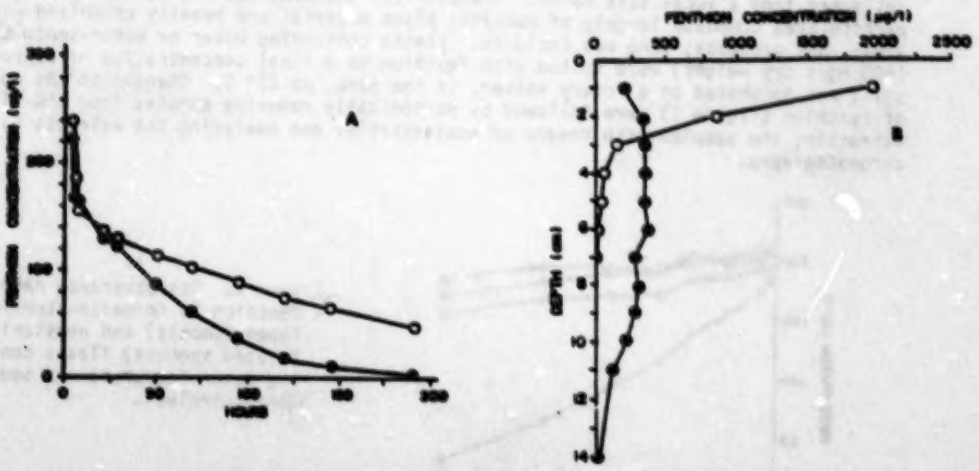

Figure 2. Concentration of fenthion in the water (A) and sediment at 170 hours (B) (open circles) and nonsterile (closed circles) saltmarsh microcosms.

Loss of the fenthion from the water column was faster in the nonsterile microcosm than in the sterile microcosm, indicating that a biotic component was controlling the fate of the pesticide. Substantial amounts of fenthion were found in the sediment bed of the nonsterile microcosms at greater depths than in the sterile microcosms. This distribution further suggests the involvement of biotic factors.

To more carefully define these biotic contributions and separate them from the other fate processes occurring simultaneously, a mathematical model, developed specifically for microcosm studies (ref. 3) was used to help interpret the data. Running the mathematical model with the appropriate kinetic parameters for diffusion (based on the movement of tritiated water), partitioning to the detrital sediment (from batch sorption tests), hydrolysis and photolysis (flask studies), showed that the fate of fenthion in the sterile microcosms could be adequately explained; i.e., good agreement between predicted and actual concentrations of the pesticide.

Similar use of the model, however, with kinetic parameters for the biodegradation of fenthion in water and sediment from the flask studies, failed to explain the fate of the pesticide in the active microcosms. This was due to a much slower rate of biodegradation and a much deeper penetration of the fenthion into the sediment bed than expected. The presence of numerous sediment-dwelling invertebrate animals in the microcosms, which are capable of extensive mixing of interstitial water and reworking of sediments (ref. 4 and 5), led to the hypothesis that such activities were responsible for the observed distribution of fenthion in the sediment bed. This bioturbation activity, coupled with the lack of anaerobic degradation of fenthion (anaerobic conditions in the microcosms occurred in the sediment at 2 cm depth and below) would explain the observed results. Rerunning the model with higher diffusion rates to simulate the bioturbation and biodegradation only in the top cm of the sediment bed projected a curve that reasonably fitted, based on chi squared analysis, the observed data. This fit is shown in figure 3 (curve D).

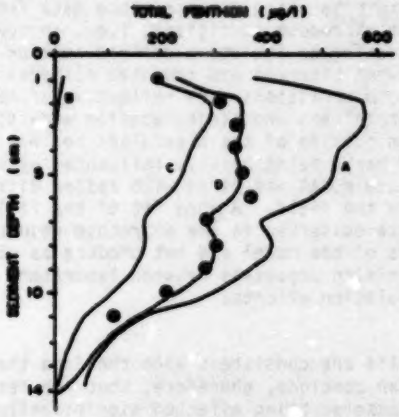

Figure 3. Concentration of fenthion with depth in the sediment of microcosms (data points) and predicted concentrations from a mathematical model (solid line).

Using biodegradation rates equal to zero (curve A), to the rate obtained from the flask studies (curve B), or to the flask reduced 40x and assumed to be equal at all depths in the sediment (curve C), gave relatively poor fits to the data. From these experiments, we have shown that a much different picture of the fate of fenthion was obtained by using experimental systems that simulate the complexity of natural systems; i.e., a variety of environmental factors can simultaneously affect the fate of a pesticide. Our experiments also suggest that biodegradation rate information from laboratory shake flask tests may not accurately reflect the rates that occur in nature.

FIELD STUDIES

To further test the importance of this information from the microcosm studies, the fate of fenthion in a field dosing experiment was considered. Fenthion, in acetone carrier, was sprayed on the water surface of the local salt marsh from which the water and sediment samples were taken for the above studies. Pesticide concentration was then monitored over time. No mechanical mixing of the marsh water was undertaken to avoid resuspending the sediments. A water-soluble dye, rhodamine WT, was also sprayed at the same time as the fenthion to measure losses due to dilution from tidal exchange. Dye concentrations were monitored by fluorescence spectrometry. Disappearance of fenthion from the marsh water was faster than the disappearance of the dye, indicating that one or more fate processes were operating at a rate which exceeded losses due to dilution. As a rough means of explaining the factors affecting the disappearance of fenthion in the field, the microcosm model was

applied to the field data. This was accomplished by assuming that the microcosm although a scaled down version of the field, replicated the essential features of that environment in all important details (i.e., that the conditions of the sediment core in the microcosm were typical of all areas of the marsh) with a flow-through condition to the washout rate of the dye in the marsh. A predicted rate of disappearance of fenthion was thus estimated from the model and compared with the field data (figure 4).

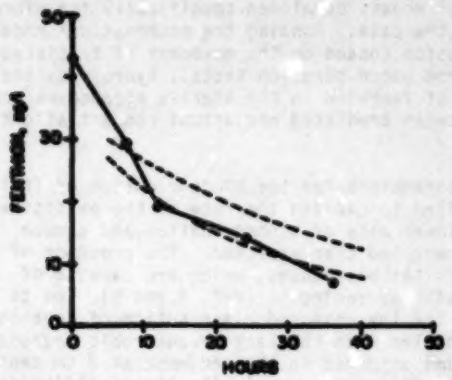

Figure 4. Actual (solid line) and predicted (dotted line) disappearance of fenthion following dosing of the water column in a salt marsh. Predicted values were based on a dilution rate calculated from dye disappearance and the kinetic parameters from the microcosm study.

As can be seen, agreement between the observed and computed disappearance curves was not close. However, in performing a similar modeling exercise using disappearance data from field conditions which more closely approximated the microcosm conditions, i.e., an open-ended plexiglass box placed in the field after the dosing to isolate a defined section of water and sediment, good agreement was obtained between observed and computed disappearance of fenthion. This result suggested that the microcosm conditions were reflective of conditions in the field: i.e., sorption, hydrolysis, photolysis and biodegradation were similar. The only major difference between the inside and the outside of the plexiglass box was the degree of water turbulence, the water mixing in the marsh being heavily influenced by wind and groundwater intrusion. Accordingly, the microcosm model was rerun with faster diffusion rates to simulate the additional water turbulence in the field. A good fit of the field data was obtained with diffusion rates that were 10x those obvserved in the microcosm results (figure 4). Changes in the other kinetic parameters of the model did not produce as good a fit. Thus, the scaling of diffusion and turbulent mixing processes between laboratory and field studies can be a crucial limitation in extrapolation efforts.

Although further field data are necessary, our results are consistent with the idea that microcosms are good surrogates for the field. We can conclude, therefore, that the fate of fenthion in a salt marsh is determined by complex factors: being affected significantly by sorption, biodegradation and bioturbation in the sediments and photolysis in the water column. Our experiments further emphasize the importance of mathematical models in studying environmental fate and the caution that should be exercised in extrapolating laboratory data to the field, particularly in the context of estimating exposure concentrations for hazard assessment procedures.

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